Effect of Early Weight-Bearing Training on Blood-Spinal Cord Barrier Function in Mice

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INTRODUCTION

Spinal cord injury (SCI) results in a breakdown of the blood-spinal cord barrier (BSCB) that permits a robust inflammatory response. Mechanisms responsible for inflammation promote further damage to the neural tissue. Neurotoxicity results from inflammatory cells moving into the spinal cord through the damaged and permeable blood vessels. Activities such as treadmill training attempt to utilize spinal plasticity to promote recovery, but recent animal studies have shown increased BSCB permeability with early swim training [1]. Exercise-regulated gelatinase matrix metalloproteinase-9 (MMP-9) is a regulator of vascular permeability utilized to degrade tight-junctions of the blood vessel wall, allowing extravasation into surrounding tissues. MMP-9 is a potent early regulator of pathology after SCI. Whether locomotor training stabilizes or exacerbates BSCB integrity is unknown.

HYPOTHESIS

Exercise delivered acutely after SCI increases hematogenous permeability at the lesion site.

METHODS

SUBJECTS & INJURY

Wild Type (WT) C57BL/6 mice (n=12) and MMP-9 null (KO) mice (n=3) received a moderate/severe contusion with the Infinite Horizon (IH) device at T9.

TRAINING PARADIGM

Manually-assisted treadmill (TM) training for 20 min occurred 2-7 days post injury (dpi). Groups were: Trained (n=5) and Untrained (n=12).

TISSUE SECTIONING

Mice were perfused with 0.1 M phosphate buffered saline followed by 4% paraformaldehyde. Tissue was collected and cryoprotected in sucrose. The lesion site was transversely sectioned (thickness = 20 um).

WHITE MATTER SPARING

Tissue was stained for myelin using eriochrome cyanine (EC). The section with the largest lesion and least amount of stained white matter represented the lesion epicenter.

ASSESSMENTS OF VASCULAR PERMEABILITY

A 2% solution of Evans Blue Dye (EBD) in normal saline was injected intravenously or intraperitoneally [2].The stain was allowed to circulate for 30 minutes before perfusion and fixation.

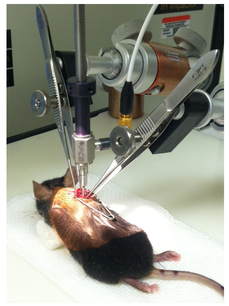
PROPORTIONAL ANALYSIS OF EBD PERMEABILITY

ImageJ was used to find the relative area of EBD penetration into the spinal cord at the epicenter and 2.4 mm both caudal and rostral to the epicenter.

CONFOCAL MICROSCOPY

The presence of EBD was detected via fluorescent confocal microscopy (Olympus FluoView FV1000) at 633 nm.

SPINAL CORD INURY METHOD

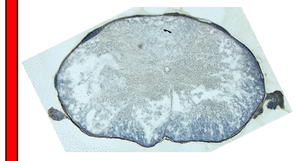


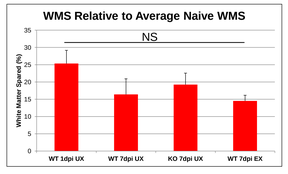
Left: Mouse stabilized in IH device with impactor positioned over exposed spinal cord.
Top: Exposed spinal cord.

EBD INJECTION METHOD

Top: Anesthetized mouse 30 min post-EBD injection.
Right: Exposed spinal cord and epicenter showing EBD presence.

WHITE MATTER SPARING





Left: Section of spinal cord at the epicenter stained with eriochrome cyanin. Dark blue indicates spared white matter.
Right: All error bars represent the standard error of the mean.

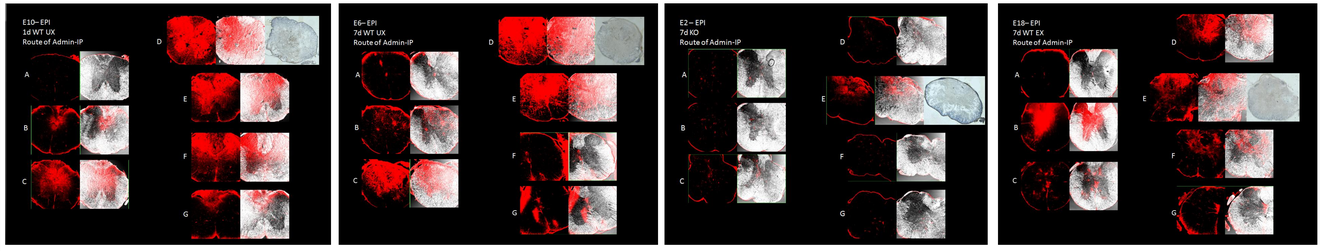
○1d WT UX showed the largest relative amount of spared white matter, while the EX group presented the least. This may show the progression of secondary pathology by 7d.

○There was little difference between KO and 7d WT UX.

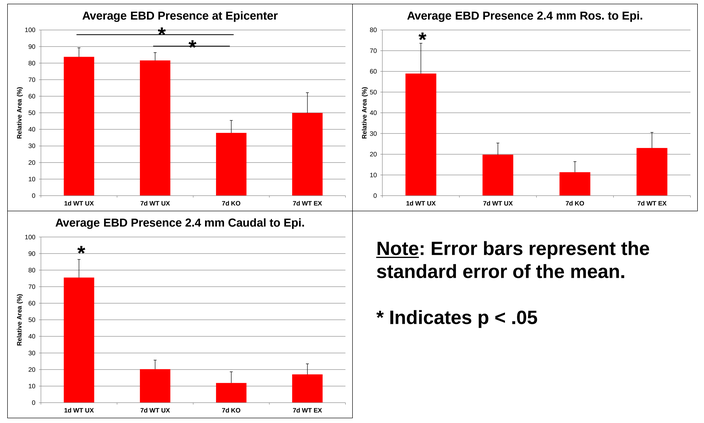
○Measure of white matter sparing did not statistically differ between groups.

HEMATOGENEOUS INFILTRATION INTO SPINAL CORD

1d WT UX Group (n=3) | 7d WT UX Group (n=4) | 7d KO UX Group (n=3) | 7d WT EX Group (n=5)



Above: Each group of images are from a single animal representative of the marked group. Paired sections are images of the same section under different microscopy settings. Red indicates fluorescence of EBD in the spinal cord tissue.



Note: Error bars represent the standard error of the mean.

* Indicates p < .05

○The data show a large degree of permeability at the epicenter in both the 1d WT UX and 7d WT UX, consistent with the section images of the representative animals. The KO and EX groups appear to show much less permeability.

○Rostral to the lesion, the greatest permeability was evident at 1d, but still present at 7d. Deletion of MMP-9 (KO) resulted in a significant reduction of Evans Blue extravasation. There was no difference between the 7d Ex and UX groups.

○1d WT UX are the most permeable caudal to the injury site. Much less permeability is evident in the 7d WT UX, 7d KO, and 7d WT EX on average. EX shows less permeability than UX.

SUMMARY AND CONCLUSIONS

○Thoracic SCI results in significant permeability within 24h that persists at 7d after SCI. Proportional assessments of Evans Blue dye reveal that 1d is notably more permeable than 7d.

○Deletion of MMP-9 reduced permeability both at and away from the lesion site. This is consistent with other work [3].

○Exercise showed a similar attenuation of BSCB permeability in WT mice. Thus, targeted locomotor training to the lumbar enlargement results in less systemic vascular demand compared to other exercise models such as swimming [1].

○It can be postulated that exercise acutely after SCI causes decreased levels of MMP-9 through modulation of MMP-9 inhibitory proteins, as previously shown with exercise prior to brain injury [4].

○Together, exercise and regulation of MMP-9 may be a novel approach to attenuate vascular events early after SCI that may influence functional recovery.

REFERENCES

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ACKNOWLEDGMENTS

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